Biochimica et Biophysica Acta 1812 (2011) 1490–1497

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Molecular machinery of macroautophagy and its deregulation in diseases

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ARTICLE INFO

Article history:
Received 30 April 2011
Received in revised form 27 June 2011
Accepted 8 July 2011
Available online 21 July 2011

Keywords:
Autophagy
Autophagosome
Cdk5
Endophilin B1
Post-translational modification
Neurodegenerative disorder

ABSTRACT

Macroautophagy maintains cellular homeostasis through targeting cytoplasmic contents and organelles into autophagosomes for degradation. This process begins with the assembly of protein complexes on isolation membrane to initiate the formation of autophagosome, followed by its nucleation, elongation and maturation. Fusion of autophagosomes with lysosomes then leads to degradation of the cargo. In the past decade, significant advances have been made on the identification of molecular players that are implicated in various stages of macroautophagy. Post-translational modifications of macroautophagy regulators have also been demonstrated to be critical for the selective targeting of cytoplasmic contents into autophagosomes. In addition, recent demonstration of distinct macroautophagy regulators has led to the identification of different subtypes of macroautophagy. Since deregulation of macroautophagy is implicated in diseases including neurodegenerative disorders, cancers and inflammatory disorders, understanding the molecular machinery of macroautophagy is crucial for elucidating the mechanisms by which macroautophagy is deregulated in these diseases, thereby revealing new potential therapeutic targets and strategies. Here we summarize current knowledge on the regulation of mammalian macroautophagy machineries and their disease-associated deregulation.

1. Introduction

Autophagy is regarded as a "self-digestion" process, which degrades a cell's own cytoplasmic content through lysosomes, for the maintenance of cellular homeostasis [1]. There are at least three types of autophagy, including chaperone-mediated autophagy (CMA), microautophagy and macroautophagy. They differ in terms of their mechanism for directing the cytoplasmic content to the lysosomes, where the engulfed content is degraded by lysosomal proteases into macromolecules and are released back into the cytoplasm [2,3]. In CMA, recognition of a specific consensus sequence on the targeted proteins by a lysosomal chaperone directs the proteins into the lysosomes; whereas in microautophagy, invagination leads to the direct engulfment of cytoplasmic content by lysosome [3]. In macroautophagy, the cytoplasmic content is enveloped into a double-membraned vesicle called autophagosome, through non-specific encircling of the bulk cytoplasm or a selective process for targeted proteins, organelles, protein aggregates and intracellular pathogens. The autophagosome then fuses with lysosome to form an autolysosome to dispose of its content. Precise regulation of macroautophagy is required to facilitate selective engulfment and degradation when needed, in addition to preventing undesired removal of cytoplasmic contents.

Macroautophagy at its optimal level ensures cell homeostasis, while its deregulation compromises cell survival [4–6]. Macroautophagy deregulation has been demonstrated in many diseases such as neurodegenerative disorders, cancers and inflammatory disorders [3,7,8]. Interestingly, the macroautophagy pathway is deregulated via distinct mechanisms in each disease. In neurodegenerative disorders, impaired macroautophagic engulfment of cytosolic content and mitochondria by autophagosomes are respectively observed in models of Huntington's disease [9] and Parkinson's disease [10–13], whereas autophagosome degradation is defective in Alzheimer's disease models [14]. In inflammatory disorder like cystic fibrosis, the machinery for autophagosome nucleation is mislocalized, which in turn inhibits macroautophagy [15]. Understanding the molecular control of macroautophagy is therefore imperative for dissecting the contribution of macroautophagy deregulation in such diverse diseases. In this review, we focus our discussion on the molecular regulation of mammalian macroautophagy, and discuss how its deregulation is linked to various diseases.

2. Molecular machinery of macroautophagy and its regulation

During the last decade, research has focused on how mammalian macroautophagy is initiated and regulated in the cell [2,16]. Since the macroautophagy machineries in organisms such as yeast, *Caenorhabditis elegans* and *Drosophila* are highly conserved with those in mammals [17–20], researchers have performed molecular and genetic screens in these organisms to identify macroautophagy regulators, leading to the

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doi:10.1016/j.bbadis.2011.07.005

subsequent identification of mammalian counterparts of these regulators [21,22]. Macroautophagy involves a battery of molecular players to initiate autophagosome formation, as well as the nucleation, elongation, maturation, and degradation of autophagosomes. Macroautophagy regulators are also modified post-translationally through ubiquitination, phosphorylation and acetylation, which facilitate the delivery of cytoplasmic contents into autophagosomes and provide an additional level of control over macroautophagy. Furthermore, distinct molecular machineries have been identified for selective targeting of certain cytoplasmic contents into autophagosomes, as well as to mediate subtypes of macroautophagy, further highlighting the complexity of macroautophagy regulation in the cell.

2.1. Initiation of autophagosome formation

The origin of autophagosome, as the starting point of macroautophagy, has been characterized as a small crescent-shaped structure called isolation membrane or phagophore. Various intracellular membranes have been suggested as the source of isolation membrane, including the endoplasmic reticulum [23], golgi apparatus [24], mitochondria [25], as well as the plasma membrane [26]. However, the mechanisms underlying the recruitment of these membranes for autophagosome formation remain unclear.

Initiation of macroautophagy involves the assembly of ULK protein complex comprising ULK1, Atg13, FIP200 and Atg101 at the isolation membranes, where this complex works with other autophagy-related gene (Atg) proteins to initiate autophagosome formation [27] (Fig. 1). The assembly of ULK complex is facilitated by a Ras-like small G protein RalB and the exocyst complex [28], and its activation requires dissociation from the negative regulator mTOR complex 1 (mTORC1) [29] during cellular stress such as starvation. Alternatively, macroautophagy can also be induced via mTOR-independent mechanisms, such as through altering the transcription of macroautophagy genes or reducing the cellular level of inositol 1,4,5-trisphosphate (IP_3) [30,31]. For example, macroautophagy is induced when IP_3 production is reduced by treatment with IP_3 receptor antagonists [32], pharmacological inhibition of inositol monophosphatase activity or inositol synthesis [33], as well as by lowering cyclic adenosine monophosphate (cAMP) level [30]. Interestingly, the reduced IP_3 level further decreases intracytosolic calcium ion concentration and calpain activity to lower the cAMP level, resulting in a feedback loop for stimulating macroautophagy [30,31]. It will be interesting to further elucidate the downstream signaling mechanism by which IP_3 inhibits macroautophagy.

Atg5 and Atg12 are also key macroautophagy regulators present on the isolation membranes [34]. Interaction of Atg5 with Atg16L1 at the isolation membranes facilitates autophagosome formation, as revealed by the observation that forced localization of Atg16L1 to the plasma membrane promotes on-site autophagosome formation [35,36]. Proteins such as phosphoinositide 3-phosphatase Jumpy and WIPI-1 are also localized to the isolation membranes [37], and further studies will elucidate how these proteins are implicated in the initiation of autophagosome formation.

2.2. Autophagosome nucleation and elongation

Autophagosome formation proceeds with nucleation and elongation of the isolation membranes to generate vesicular structures [2,38] (Fig. 1). Nucleation of isolation membranes requires the formation of a large protein complex, known as Beclin 1/Class III phosphatidylinositol-3-kinase (PI3K) complex, coordinated by the interactions of several proteins including Beclin 1, UV irradiation resistance-associated tumor suppressor gene (UVRAG), Atg14, B-cell leukemia/lymphoma-2 (Bcl-2), p150, ambra1, endophilin B1, and PI3K Vacuolar protein sorting 34 (Vps34), which then activates PI3K to produce phosphatidylinositol-3-phosphate [2,31]. Beclin 1/PI3K complex formation and PI3K activity are inhibited when Beclin 1 is bound to Bcl-2 [39], but are stimulated upon UVRAG recruitment to the complex [40]. Ambra1 also directly binds Beclin 1 to regulate Beclin 1/PI3K complex formation [41]. Interestingly, Atg14 and UVRAG are located in the Beclin 1-PI3K complex in a mutually exclusive manner [42] and Atg14 competes with UVRAG for Beclin 1 to promote autophagosome formation [43]. These findings suggest a coordinated role of specific components within the Beclin 1/P13K complex to drive nucleation of isolation membranes. Notably, endophilin B1, also known as Bax-interacting factor 1 (Bif-1) [44] or SH3GLB1 [45], is hitherto the only N-terminal Bin-Amphiphysin-Rvs (N-BAR) domain-containing protein identified in this Beclin 1/PI3K complex, and is believed to generate membrane curvature during vesicle nucleation [46]. The function of endophilin B1 in macroautophagy regulation requires both its N-BAR domain and Src-homology 3 domain, with the latter shown to mediate its interaction with UVRAG to recruit Beclin 1 and facilitate PI3K activation [47]. Endophilin B1 was also reported to have a role in coordinating the intracellular trafficking of another macroautophagy-related membrane protein Atg9 [48]. Additional proteins such as PTEN-induced putative kinase 1 (PINK1) [49], death-associated protein kinase (DAPK) [50], IP_3 receptor [32] and high mobility group box 1 [51] have also been identified as Beclin 1-binding protein and marcoautophagy regulator. How they work with other partners in the Beclin 1/PI3K complex to direct membrane nucleation are to be further examined.

Following the nucleation step, other Atg proteins are recruited to the membrane of the pre-autophagosomes to promote elongation and expansion, and eventually the completion of autophagosome formation [2,38] (Fig. 1). During the elongation and expansion steps, Atg7 and Atg10 facilitate the formation of a covalently-linked Atg5–Atg12 complex [52], which also interacts with Atg16L1 [35]. Together with Atg3, Atg4 and Atg7, the Atg5–Atg12–Atg16L1 complex mediates the conjugation of phosphatidylethanolamine (PE) to the microtubule-associated protein 1 light chain 3 (LC3)-I to form LC3-II, leading to the translocation of LC3 from cytoplasm to the membrane of the pre-autophagosomes [34–36,53]. Once autophagosome formation is completed, the Atg proteins are released back to the cytoplasm by a yet uncharacterized mechanism.

2.3. Autophagosome maturation and degradation

Subsequent to the elongation step, autophagosomes are fused with lysosomes for degradation of their contents, a process known as autophagosome maturation [38,54,55]. Autophagosomes fuse with several types of vesicles from the endosomal/lysosomal pathways including late endosomes and lysosomes [54]. Consistently, autophagosome maturation and degradation require the action of late endosome marker protein Rab7 and lysosomal membrane protein LAMP-2 [56,57]. The molecular mechanisms governing autophagosome maturation remain unclear, but recent studies have identified new regulators of autophagosome maturation and degradation such as UVRAG [58], Rubicon [59,60], presenilin-1 [14], valosin containing protein (VCP) [61], and syntaxin-5 SNARE complex proteins [62]. UVRAG interacts with the class C vacuolar protein sorting complex, a key machinery for endosomal fusion, and enhances Rab7 activity for promoting autophagosome fusion with late endosomes and lysosomes [58]. Interestingly, interaction of UVRAG with Rubicon negatively regulates autophagosome maturation [59,60]. On the other hand, inhibition of presenilin-1 [14], VCP [61] or syntaxin-5 SNARE complex [62] impairs lysosomal degradation of autophagosomes. Knowing how these molecules function and work together would provide a clearer picture of the maturation and degradation process.

2.4. Post-translational control of macroautophagy regulators

Regulation of macroautophagy further involves post-translational modifications of macroautophagy-related proteins (Fig. 1). In particular, phosphorylation often modulates the function of macroautophagy regulators that are implicated in the initiation of macroautophagy and

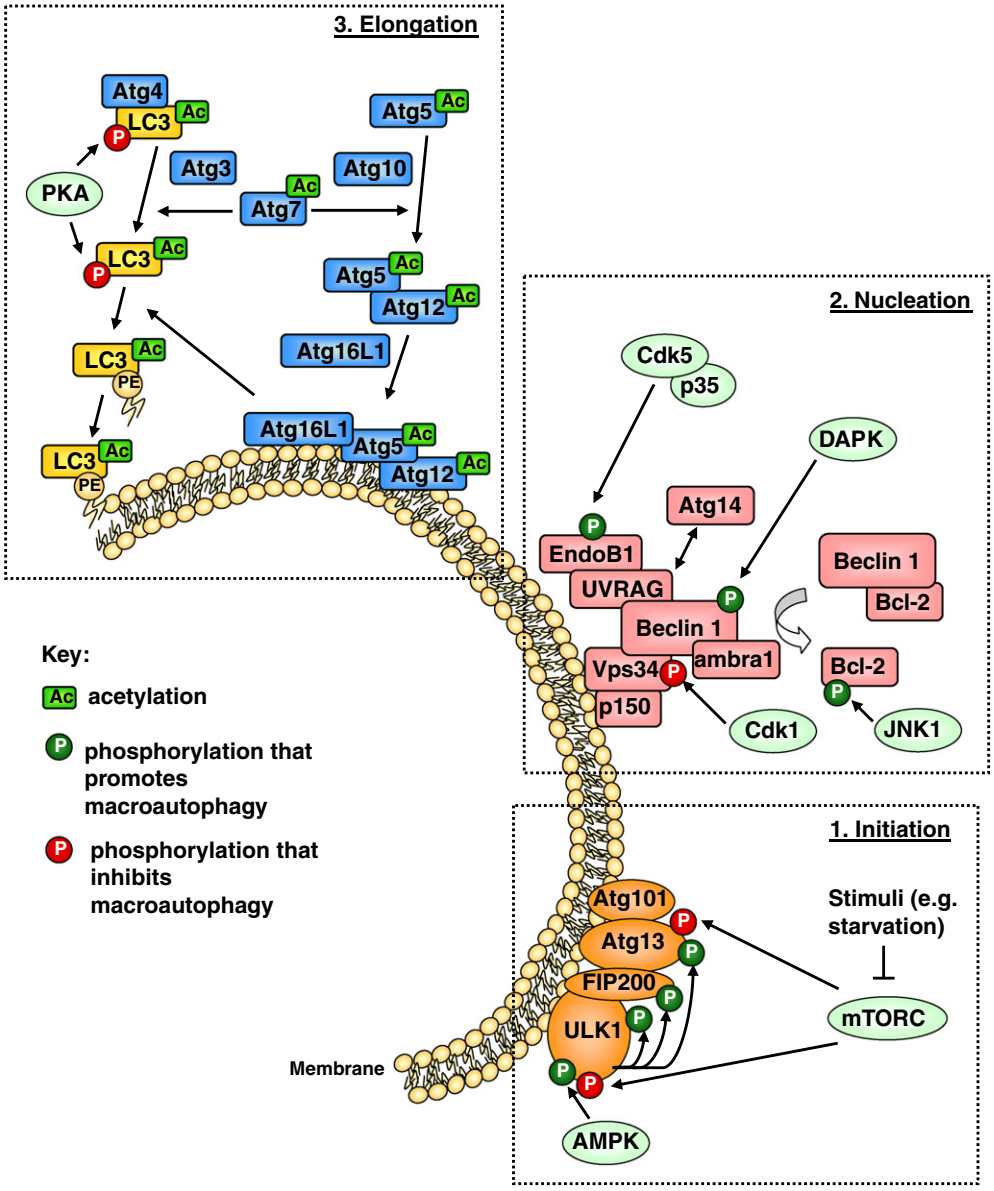

Fig. 1. Molecular regulation of autophagosome formation in mammalian macroautophagy. Macroautophagy begins with the assembly of molecular players at isolation membrane to initiate autophagosome formation, followed by nucleation and elongation of the membranes. The ULK protein complex comprising ULK1, Atg13, FIP200 and Atg101 dissociates from mTORC1 and assembles at the isolation membrane to initiate autophagosome formation during cellular stimuli such as starvation. Membrane nucleation involves the formation of Beclin 1/PI3K complex, coordinated by the interactions between Beclin 1, UVRAG, Atg14, Bcl-2, p150, ambra1, endophilin B1 and Vps34. Elongation of membranes requires Atg3, Atg4, Atg7, Atg10, and an Atg5–Atg12–Atg16L1 complex to conjugate PE to LC3, leading to the translocation of LC3 from cytoplasm to the membrane of the forming autophagosomes. Phosphorylation and acetylation of macroautophagy machinery proteins provide additional control over autophagosome formation.

the formation of autophagosomes. For example, initiation of macroautophagy requires ULK1 phosphorylation by adenosine monophosphate-activated protein kinase (AMPK), while ULK1 phosphorylation by another kinase mTOR, a component of mTORC1, inhibits macroautophagy through its regulation of ULK1/AMPK interaction [63–65]. On the other hand, phosphorylation of Bcl-2 by c-Jun N-terminal protein kinase 1 [66] and that of Beclin-1 by DAPK [50] reduce the inhibitory binding of Bcl-2 to Beclin 1 to increase autophagosome formation. Vps34 phosphorylation mediated by cyclin-dependent kinase 1 decreases the binding affinity of Vps34 to Beclin 1, thereby reducing macroautophagy induction [67]. Protein kinase A-dependent phosphorylation of LC3 was also shown to reduce the formation of autophagosomes [68]. In addition, we have recently demonstrated that phosphorylation of endophilin B1 by cyclin-dependent kinase 5 (Cdk5), a proline-directed serine/threonine kinase with a predominantly neural-specific activity, facilitates the interaction of endophilin B1 with UVRAG and Beclin 1, leading to macroautophagy induction in neurons [69]. All these findings underscore the importance of protein phosphorylation in controlling the initiation of macroautophagy and autophagosome formation in the cell.

Acetylation of macroautophagy regulators presents another mechanism by which macroautophagy is modulated. For example, acetylation status of several Atg proteins including Atg5, Atg7, LC3, and Atg12 is inversely correlated with the cellular level of macroautophagy [70,71] (Fig. 1). Knockdown of acetyltransferase p300 reduces acetylation of Atg5, Atg7, LC3 and Atg12 and enhances macroautophagy, while overexpression of p300 increases acetylation of these Atg proteins and inhibits macroautophagy [71]. Furthermore, acetylation of Atg5 is markedly elevated in mice lacking deacetylase Sirtuin-1, which exhibit macroautophagy-deficit phenotypes such as accumulation of protein

aggregates [70]. This phenotype is similar to those observed in Atg5 knockout mice [72]. Regulating the acetylation status of macroautophagy-related proteins thus modulates the level of macroautophagy in the cell. In addition, proteolytic processing of macroautophagy proteins has emerged as another regulatory mechanism of macroautophagy since both cleavage of Beclin 1 by caspase [73] and that of Atg5 by calpain [74] reduces macroautophagy.

Recent studies revealed that post-translational modifications of macroautophagy regulators also take part in conferring selectivity in the macroautophagy of certain cytoplasmic content such as protein aggregates, organelles, and intracellular pathogens. In particular, ubiquitination has emerged as an important mechanism for mediating selective degradation of protein aggregates through macroautophagy [75–78]. Ubiquitinated protein aggregates are recognized by p62 and NBR1 that deliver the aggregates to autophagosomal membranes [79–81]. Autophagy-linked FYVE protein (Alfy), an Atg5-interacting protein, is further recruited to p62-positive protein aggregates and facilitates their macroautophagic degradation through scaffolding the interaction between the aggregates and Atg5 [82,83]. To selectively target damaged mitochondria for macroautophagic degradation, a process known as mitophagy, ubiquitin E3 ligase Parkin is translocated from cytoplasm to the damaged mitochondria through the action of PINK1 [10–12,84]. It was further reported that Parkin ubiquitinates the mitochondrial protein Voltage-dependent anion channel 1 (VDAC1), which recruits p62 and directs the damaged mitochondria into autophagosomal membranes for subsequent macroautophagic degradation [12]. Nonetheless, it should be noted that another research group failed to detect the involvement of VDAC1 and p62 in this process [85]. Another intriguing observation is that Parkin promotes mitophagy in PINK1-deficient cells [86], suggesting that Parkin may also trigger mitophagy in a PINK1-independent manner. Additional proteins such as Nix [87,88] are required for mitophagy of damaged mitochondria, but the mechanisms involved remain unclear. Ubiquitination also facilitates the macroautophagic degradation of intracellular pathogens. For example, intracellular bacteria coated with ubiquitin are recognized by the ubiquitin-binding protein NDP52 and are then delivered for macroautophagic degradation, a process called xenophagy [89]. These findings collectively underscore the importance of ubiquitination in selecting substrates for macroautophagic degradation. Another post-translational modification demonstrated to facilitate selective targeting of cargo for macroautophagy is acetylation. Upon acetylation, the aggregation-prone mutant huntingtin proteins are delivered to autophagosomes, resulting in increased clearance through macroautophagy [90]. Precise regulatory mechanisms are therefore present in the cell to facilitate selective degradation of cytoplasmic contents through macroautophagy to prevent any undesired removal.

2.5. Subtypes of macroautophagy

Subtypes of macroautophagy that are independent of Atg5 and Atg7 [91] or Beclin 1 [92–95] have been reported. The regulation of Atg5/Atg7-independent macroautophagy involves ULK1, Beclin 1, and an endosome-Golgi transport protein Rab9 [91], whereas Beclin 1-independent macroautophagy has been demonstrated to bypass the requirement for PI3K [96]. Further investigations are required to understand the molecular details of these subtypes of macroautophagy. Interestingly, recent evidence suggests that "basal" and "stress-induced/pathological" macroautophagy may emerge as two subtypes of macroautophagy that possibly involve distinct sets of macroautophagic machinery. For example, in rat neurons, endophilin B1 phosphorylation by Cdk5 is required for macroautophagy and neuronal death induced by several stress- or death-inducing stimuli, but is dispensable for basal macroautophagy [69]. Furthermore, an engulfment factor Draper functions as a regulator of macroautophagy during cell death, but is not involved in starvation-induced macroautophagy that are associated with survival in *Drosophila* cells [97]. These findings suggest that "stress-induced/pathological" macroautophagy may recruit a unique set of macroautophagy regulators. The possible presence of distinct macroautophagic machineries for "basal" and "stress-induced/pathological" macroautophagy raises the possibility that they may indeed be two subtypes of macroautophagy. It will be important to further dissect the signaling pathways that are implicated in determining the subtypes of macroautophagy activated in the cells.

3. Deregulation of macroautophagy in diseases

Macroautophagy is crucial for cell survival in stressful conditions and for maintenance of cellular homeostasis [98]. Increasing evidence indicates that macroautophagy machineries are deregulated in diverse human diseases, including neurodegenerative disorders, cancers and inflammatory disorders [3,7,8,99]. Notably, macroautophagy is deregulated at distinct steps including autophagosome nucleation, maturation and degradation in each of these diseases.

3.1. Neurodegenerative disorders

Neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and various spinocerebellar ataxias are characterized by pathologies including neuronal dysfunction, neuronal death and accumulation of disease proteins [100,101]. Excessive activation of macroautophagy or defects in autophagosome maturation and degradation has been described in these disorders and is tightly associated with disease pathology.

Mutations in presenilin-1 that cause early-onset AD have been associated with defective lysosomal degradation of autophagosomes [14]. Moreover, impaired lysosomal degradation selectively disrupts axonal transport of autophagosomes, causing their accumulation within the dystrophic axons [102]. Since autophagosomes are enriched with amyloid-beta (Aβ) precursor protein and its processing enzymes for generating toxic Aβ [103], the accumulation of autophagosomes caused by impaired lysosomal degradation in AD may represent a source of Aβ that contributes to neurodegeneration [103]. Supporting this idea, autophagosome accumulation is detected in the brain of AD patients [104]. Genetically ameliorating the impaired macroautophagic and lysosomal degradation in an AD mouse model reduces Aβ accumulation and memory deficits [105]. Nevertheless, decreased Beclin 1 expression is also detected in AD brains, whereas Beclin 1-deficient mice display decreased macroautophagy but increased neurodegeneration [106]. Further studies will elucidate the association between macroautophagy deregulation and AD pathogenesis.

Multiplication of alpha-synuclein (α-syn) gene, and thus its increased expression, is one of the genetic causes of PD. While α-syn overexpression reduces the level of Atg7 and impairs macroautophagy [107,108], viral transfer of Atg7 or Beclin 1 gene, or infusion of rapamycin, increases macroautophagy and ameliorates neurodegenerative phenotypes in mice overexpressing α-syn [107,109]. Conversely, macroautophagy is induced in neurons expressing PD-associated A53T mutant α-syn proteins [110–112]. Genetic knockdown of Atg5, Atg12 or endophilin B1 inhibits neuronal death in A53T α-syn-expressing neurons, indicating that macroautophagy is involved in induced neuronal loss in this disease context [69,110]. Furthermore, PD-associated mutations in Parkin [10–12,113] and PINK1 [13] interfere with their functions in promoting mitophagy of damaged mitochondria, the level of which influences cell survival [86]. The extent of macroautophagy is also altered in cells deficient in Leucine-rich repeat kinase 2 (LRRK2) [114,115], with the PD-linked mutation in LRRK2 increasing autophagosome level [116]. Further studies will define how LRRK2 and its mutations contribute to neurodegeneration through deregulating macroautophagy.

Macroautophagy degrades expanded polyglutamine-containing disease proteins found in HD and spinocerebellar ataxias, as well as PD-associated A53T α-syn proteins [117,118]. It was recently revealed that in model of HD, engulfment of cytosolic content by autophagosomes is

defective despite having a normal rate of autophagosome formation [9]. The disruption of axonal transport in HD [119,120] and the reduced level of lysosomes in PD models [121,122] may also contribute to inefficient degradation of autophagosomes in neurons. Although it remains controversial as to whether the accumulation of disease proteins mediates neuronal toxicity or serve a protective function [123], clearance of disease proteins found in HD and spinocerebellar ataxias by pharmacological activation of macroautophagy is often accompanied by an alleviation of neurodegenerative phenotypes [30,124,125], suggesting a protective role of macroautophagy in pathogenesis of HD and spinocerebellar ataxias.

3.2. Cancers

A number of macroautophagy-related genes have been reported to be mutated or downregulated in cancers. Beclin 1 is mono-allelically deleted in human breast, ovarian and prostate cancers, and the expression of Beclin 1 is reduced in cancers, including those in breast, ovary and the brain [126,127]. In addition, mice lacking Beclin 1 have a high incidence of spontaneous tumor formation [128,129], while gene transfer of Beclin 1 inhibits the tumorigenicity of breast cancer cells in mice and the growth of breast cancer cell lines [126]. Restoration of Beclin 1 expression also inhibits the growth of colon cancer cells [130]. These findings collectively demonstrate that decreased expression of Beclin 1 leads to tumorigenesis.

Similar to mice deficient in Beclin 1, endophilin B1 null mice also form spontaneous tumors at a high frequency, indicating a tumor-suppressive function of endophilin B1 [47]. Mutation in endophilin B1 is rare in cancers, with only one somatic mutation detected in lung carcinoma [131]. Nonetheless, decreased expression of endophilin B1 is common in various cancers including colorectal adenocarcinoma and gastric cancers, as well as in subsets of urinary bladder, gallbladder and prostate cancers [132–135]. UVRAG is another macroautophagy regulator that suppresses the proliferation of human colon cancer cells [40]. Mutations in UVRAG are frequently detected in colon cancers [136], and are also observed in gastric cancers [137].

It is hypothesized that the tumor-suppressive functions of Beclin 1, endophilin B1 and UVRAG, the three components of the Beclin 1/PI3K complex, are attributed to their roles in autophagosome nucleation, and/or other functions such as the control of cell division [138]. Nevertheless, it was reported that colon cancer-associated mutations in UVRAG do not affect its function in macroautophagy [139]. Mutations in other macroautophagy genes such as Atg5 are also detected in gastric and colorectal cancers [140]. Further studies will define whether these mutations contribute to tumorigenesis through affecting macroautophagy.

3.3. Inflammatory disorders

Deregulation of macroautophagy occurs in several inflammatory disorders. In cystic fibrosis, a chronic inflammatory disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, mutations in CFTR result in mislocalization of the Beclin 1/PI3K complex and inhibit macroautophagy in lung epithelial cells [15]. Importantly, restoring the expression of Beclin 1 or the level of macroautophagy alleviates the cystic fibrosis disease phenotypes [15], suggesting a detrimental role of deregulated macroautophagy in the pathogenesis of cystic fibrosis.

Macroautophagy directs intracellular pathogens including bacteria into autophagosomes for lysosomal degradation. Interestingly, Crohn's disease, a chronic inflammatory disorder of the small intestine, exhibits impaired bacterial clearance and susceptibility to the disease is increased in patients with mutations in three marcoautophagy-related genes NOD2, Atg16L1 and IRGM [99]. Crohn's disease-associated NOD2 risk variants-expressing cells show defects in macroautophagy induction [141] and aberrant engulfment of bacteria into autophagosomes [142], supporting a link between macroautophagic deregulation and Crohn's disease. Conversely, despite the reported functions of Atg16L1 [35] and IRGM [143] in macroautophagy regulation, it remains to be clarified whether the Crohn's disease-associated risk variants contribute to bacterial clearance and disease pathology through altering their functions in macroautophagy regulation [99].

3.4. Macroautophagy deregulation in other diseases

Deregulated macroautophagy has been reported in diseases such as inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD), centronuclear myopathy and Pompe disease. Notably, IBMPFD-associated mutations in VCP impair its function in autophagosome maturation and degradation [61,144], while a mutation in Jumpy found in centronuclear myopathy also disrupts its regulation of macroautophagy [37]. In addition, impaired autophagosome maturation is implicated in Pompe disease pathology [145], with the disease pathology alleviated in macroautophagy-deficient mice following therapy [146]. It would be interesting to understand how macroautophagy contributes to the pathologies of these diseases.

4. Conclusions

It is evident that deregulation of macroautophagy occurs in various diseases, and disease-associated pathologies can be alleviated by modulating the level of macroautophagy. Nonetheless, each disease involves deregulation of macroautophagy at distinct steps such as autophagosome nucleation, maturation and degradation. Substantial progress has been made in the past decade to decipher the molecular mechanisms governing macroautophagy, and we are beginning to understand how macroautophagy is selectively and differentially regulated under various physiological and disease conditions. Elucidating the molecular mechanisms underlying the precise regulation of macroautophagy will shed light on novel therapeutic targets or strategies for these diseases.

Acknowledgements

We apologize to authors whose work could not be discussed or cited due to space limitations. This work was supported in part by the Research Grants Council of Hong Kong (HKUST 661007, 661109, 660309, 660210, and 6/CRF/08), the Area of Excellence Scheme of the University Grants Committee (AoE/B-15/01) and the Hong Kong Jockey Club. N.Y.I. and Z.H.C were recipients of the Croucher Foundation Senior Research Fellow and Croucher Foundation Fellow respectively.

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